# 0. General Notes

**Important Note:** From July 2017, you must agree to the Analytics Core Facility Essen (ACE) Terms of Use if you want to use our services. The Terms of Use can be found on the ACE website (<https://www.uni-due.de/ace>).

**Important Note:** From July 2017, ACE services are no longer free of charge. The current price list can be found on the ACE website (<https://www.uni-due.de/ace>).

# 0.1. Requirements for Sample Submission

Use of our services requires that you provide a set of documents:

1. First-time users need to provide a duly filled and signed “ACE Registration Form” to the organizational manager of the ACE (scanned email attachment is sufficient). Please note that the document requires a signature by the user and his/her PI (principle investigator, group leader). By this procedure, you acknowledge acceptance of our “ACE Terms of Use”.

2. A duly filled “Sample Submission Form (SSF)” (please always use the current version) for every project. This document has to be sent to the organizational manager of the ACE **before you start** your sample preparation. Please provide all requested information; particular care should be given to Section 1.6. and Section 1.8 (detailed experimental scheme/design).

3. The SSF is evaluated by the organizational manager of the ACE. Upon acceptance of your SSF, you and your PI will receive a return email containing your SSF with an updated Project number and a separate letter with the estimated costs for the project. The costs **are estimated** from the information in your SSF (e.g. number of samples given in Section 1.6.).
If the SSF is inadequate (e.g. because of inappropriate experimental design or missing data/information), you will be asked to clarify these issues and to resubmit an improved SSF.

After fulfillment of points 1 to 3, you may send your samples to us.

## 0.2. Send the samples to:

Universität Duisburg-Essen

Analytics Core facility Essen

c/o Dr. Farnusch Kaschani

Universitätsstr. 2

45117 Essen

**Important Note:** Make sure your samples are labelled with the unique ACE ID’s (see below for details).

## 0.3. How long will the analysis take?

This depends on many factors, e.g. the complexity of your experiment, residual capacities at the ACE and so on … Nevertheless, “internal” samples (e.g. from the Faculty of Biology or ZMB) are treated with priority. We aim to process a set of samples within 2 weeks. The subsequent data analysis however is usually rather time consuming and strongly depends on the complexity of the experiment; this may take another 2 to 6 weeks.

Please note that unexpected LC or MS device downtimes may result in significantly extended processing times. We usually do not send out status notifications but feel free to contact us in urgent cases.

## 0.4. What you can expect:

We will process your samples according to your experimental needs. Our lab offers a broad range of MS experiments and sample preparation techniques. Please let us know what you need.

We try to be as transparent as possible with respect to data analysis. You will receive a detailed account of all performed analysis steps. An overview is attached to this file. Nevertheless, you will have to evaluate our findings. We will make suggestions (and these are only suggestions) what should be done after your analysis.

There is one thing though which we will not do: **We will not hand out RAW data!**

## 0.5. Rules for proper sample preparation

In most cases you will want us to perform some kind of mass spectrometry experiment on your samples. Mass spectrometry is extremely sensitive. Low femtomol (10-15 mol, down to amol, 10-18 mol) amounts of a compound can be detected by a good machine. If your samples are however overloaded with contaminants, you will end up with measuring only those and fail to detect the desired analyte. It is, therefore, essential to follow a couple of important rules.

**Rule 1:** Always wear powder free gloves to reduce the chance of contaminating your samples with keratin. Keratin is a component of your skin.

**Rule 2:** Never wear cloths made from animal wool while handling your samples. And yes, cashmere is not only a province in Pakistan/India, it is also a type of wool made from cashmere goats. As well as skin, hair also contains keratin. If pieces of this find their way into your sample, this will compromise your MS results. Therefore, please make sure that your hair never gets in contact with your samples.

**Rule 3:** Make sure that your glass ware is MS clean. MS clean means that in the best case your glass ware is either absolutely new or it was never in contact with any type of cell culture media. Tip: We label MS glass ware in the lab with a yellow tag. Nobody is allowed to use this glass ware for media preparation and nobody is allowed to touch this glass ware without gloves.

**Rule 4:** Use MS water for making stocks. MS water means very pure water, water sources that other people usually do not touch. You will compromise your MS results if you make your stock solutions with water from a doubtful source. A doubtful source is a MilliQ device where people touch the tab with their bare fingers (no gloves). They will contaminate the tab with keratin which will accumulate during your experiment and may have a negative impact on your MS analysis.

**Rule 5:** Please never autoclave solutions you intend to use in a proteomics pipeline. Auto­claving solutions mobilises plasticisers and other contaminants which will “pollute” your solution and, thereby, your samples. This is particularly problematic if you use such a solution in washing steps. The contamination will accumulate.

**Rule 6:** Plasticisers are a big problem when working with plastic ware. Low binding plastic ware is acceptable. However, at all times avoid cutting or scratching the plastic ware. At the cut sites plasticisers are exposed. When these parts come in contact with water, acetonitrile or other organic solvents they will be washed out and contaminate your samples. This will interfere with your downstream MS analysis.

**Rule 7:** Do not use working solutions over a longer period of time. Contaminants may accu­mulate which may compromise your results. Make new working solutions before starting a new experiment.

**Rule 8:** Never use spatulas, spoons, pipettes, magnetic stirrer bars or any other equipment that is usually used by others to make cell culture media or which is touched by others with their bare hands. Try to keep any equipment that’s being used in the proteomics pipeline separate from other work being done in the lab.

**Rule 9:** Wherever possible avoid using detergents. Detergents have the nasty property to accumulate during sample preparation for MS (they stick to C18). They will then elute with your sample and suppress ionization of your analyte. Also, detergents will built up in the mass spectrometer as they are not volatile. This will decrease the performance of the MS in the long run. Therefore, take special care to properly wash samples before in-gel digestion (SDS removal).

## 0.6. List of acceptable consumables and chemicals

Here you will find a table with consumables that are acceptable for preparation of MS samples. If you prepare samples for MS and you want us to run the samples we encourage you to use only the specified consumables and chemicals.

|  |  |  |
| --- | --- | --- |
| Product Name | Producer | Product Nr. |
|  Tubes |   |   |
| Protein LoBind Tube 0.5 mL | Eppendorf | 0030 108.094 |
| Protein LoBind Tube 1.5 mL | Eppendorf | 0030 108.116 |
| Protein LoBind Tube 2.0 mL | Eppendorf | 0030 108.132 |
| Protein LoBind Tubes 5.0 mL | Eppendorf | 0030 108.302 |
| Eppendorf Falcon Tubes V=15mL | Eppendorf | 0030 122.151 |
| Eppendorf Falcon Tubes V=50mL | Eppendorf | 0030 122.178 |
|  Tips |   |   |
| GELoader Tips Reloads 0.5 - 20µL | Eppendorf | 0030 001.222 |
| ep.T.I.P.S. LoRetention Reloads 2 - 200µL | Eppendorf | 0030 072.065 |
| ep.T.I.P.S. LoRetention Reloads 0.1 - 10µL | Eppendorf | 0030 072 .049 |
| ep.T.I.P.S. LoRetention Reloads 50 - 1000µL | Eppendorf | 0030 072.073 |
| TIPS 500µL - 5000µL | Eppendorf | 0030 000.978 |
| TIPS 1mL - 10mL | Eppendorf | 0030 075.145 |
| Plates |  |  |
| Microplate 96/V-PP | Eppendorf | 0030 061.300 |
| Deepwell Mat 96 | Eppendorf | 0030 127.552 |

|  |  |  |  |
| --- | --- | --- | --- |
| chemical name | Alias | Supplier | Product Nr. |
| Acetonitrile, LC-MS Ultra CHROMASOLV®, tested for UHPLC-MS | ACN | Sigma Aldrich | 14261-1L |
| Acetic Acid Optima LC/MS  | AcOH | Fisher Scientific | A113-50 |
| Acetonitrile (ACN, Optima, LC-MS, 99.9%)  | ACN | Fisher Scientific | 10489553 |
| Acetonitrile LC-MS Ultra Chromasolv® | ACN | Sigma Aldrich | 14261-1L |
| Acetonitrile Optima® LC/MS | ACN | Fisher Scientific | A955-1 |
| Acrylamid/Bis - Lösung 37,5:1 (30%m/v) | H2O | Serva Electrophoresis | 10688.02 |
| Agarose NA |  | GE Healthcare | 17-0554-02 |
| Ammoinium bicarbonate | ABC | Sigma Aldrich | 11213-1KG-R |
| Ammonium Acetate |  | Sigma Aldrich | A1542-500G |
| DL-Dithiothreitol  | DTT | Sigma Aldrich | D9779-5G |
| Formaldehyde solution |  | Sigma Aldrich | F8775-25ml |
| Formaldehyde-13C, d2 solution |  | Sigma Aldrich | 596388-5G |
| Formaldehyde-d2 solution |  | Sigma Aldrich | 492620-20G |
| Formic acid (FA, Optima, LC-MS, >99.5%)  | FA | Fisher Scientific | 10596814 |
| Formic Acid Optima LC/MS | FA | Fisher Scientific | A117-50 |
| Methanol LC-MS Ultra Chromasolv® | MeOH | Sigma Aldrich | 14262-2L |
| N,N,N',N'-Tetramethylethylenediamine | TEMED | Sigma Aldrich | T9281-25ml |
| Propan-2-ol Optima® LC/MS Grade | i-PrOH | Fisher Scientific | A461-1 |
| Sodium Cyanoborhydride |  | Sigma Aldrich | 156159-10G |
| Sodium Cyanoborodeuteride |  | Santa Cruz Biotechnology | sc-258163 |
| Sodium Cyanoborodeuteride 96 atom % D, 98% (CP) |  | Sigma Aldrich | 190020-1G |
| Sodium dodecyl sulfate, ReagentPlus® | SDS | Sigma Aldrich | L4509-10G |
| Thiourea |  | GE Healthcare | RPN6301 |
| Tris(2-carboxyethyl)phosphine hydrochloride  | TCEP | Sigma Aldrich | C4706-2G |
| Tris(hydroxymethyl)aminoethane PlusOne | Tris | GE Healthcare | 17-1321-01 |
| Urea PlusOne | Urea | GE Healthcare | 17-1319-01 |
| Wasser HiPerSolv Chromanorm für HPLC LC/MS Grade | H2O | VWR | 83,645,320 |
| Wasser LC-MS Ultra Chromasolv® | H2O | Sigma Aldrich | 14263-1L |
| Water (H2O, Optima, LC-MS, 99.9%)  | H2O | Fisher Scientific | 10728098 |

|  |  |  |  |
| --- | --- | --- | --- |
| Proteases | Alias | Supplier | Product Nr. |
| Chymotrypsin, Sequencing Grade, 4 x 25ug | Chym | Promega | V1062 |
| Glu-C, Sequencing Grade, 5 X 10ug | Glu-C | Promega | V1651 |
| Trypsin, Mass Spec Grade, 5 × 20µg | Trypsin | Promega | V5113 |
| Lysyl Endopeptidase, MS Grade | Lys-C | Wako | 125-05061 |

 **ACE Nr.: ACE\_\_\_\_\_**

 **Receive date: \_\_.\_\_.\_\_\_\_**

 **stored in box: #\_\_\_\_\_**

# 1. Sample Submission Form

## 1.1. Project name

(Provide a descriptive title e.g. “Effect of Toxin X on *A. thaliana* proteome”)

Your text

## 1.2. Your contact details

### Contact details: Submitter

|  |  |
| --- | --- |
| **Name** |  |
| **Institute** |  |
| **Group** |  |
| **Address:** |  |
| **City:** |  |
| **Zip code:** |  |
| **Country:** |  |
| **Phone number** |  |
| **Email**  |  |

### Contact details: Group Leader

|  |  |
| --- | --- |
| **Name** |  |
| **Institute** |  |
| **Address:** |  |
| **City:** |  |
| **Zip code:** |  |
| **Country:** |  |
| **Phone number** |  |
| **Email** |  |

## 1.3. Experiment background

Please indicate in this section why you performed this experiment, what answers you expect and how you designed your experiment. Also briefly indicate why you want MS done on your samples (e.g. I would like to investigate what the effect of a toxin is on the proteome composition of Arabidopsis leaf proteome. I expect that the abundance of certain proteins is reduced upon toxin treatment. I treated plants with the toxin and with a control. I took samples at two time points. Now I would like to compare the protein composition at the different time points using quantitative mass spectrometry).

Your text ---keep it short!

## 1.4. Sample Buffers

Please indicate the composition of the buffers and solutions your samples have come in contact with. We need the full composition. Do not just write PBS 🡪 find out what the exact composition is and mention the molarity of the individual components here. For very complex buffers submit a separate word file e.g. from the manufacturer. Give a unique Buffer ID to each buffer. Please note the format: B#\_”variable capital letter”. In most cases you will have only 2 to 3 Buffers (the buffer you grew your cells in, the buffer you extracted your proteins in and maybe the buffer you submit digested proteins in). Don’t mention wash buffers unless they contain detergents.

|  |  |
| --- | --- |
| **Buffer ID/ name** | **Composition** |
| B#\_A | 50 mM Tris pH 7.4 |
| B#\_B | DMEM, high glucose cell culture medium; Complex composition. See additional file 🡪 „file name“ (must be provided) |
| B#\_C | 0.1 % formic acid  |
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Add rows if necessary

## 1.5. Chemicals used during sample preparation

Please indicate in this section which chemicals, detergents, salts … the samples have seen starting from the beginning of the experiment. We need this information to assess if any of the compounds are a danger for the mass spectrometer or will have a negative influence on the analysis. List the compounds one after another delimitated by a comma. Please provide concentrations if known. And explain abbreviations. Do not mention buffer components as they should feature in the previous buffer section.

Your text.

## 1.6. Sample descriptions

The ACE ID must follow the format: XX## (e.g. FK01 for **F**arnusch **K**aschani Sample **01**). Internally we will only use the ACE ID!!! Also, when we write reports we will only use the ACE ID’s. At this point provide a general overview of your samples and the experimental setup. What organ did you use, what organism does it come from? What are the different treatments? How was the sample generated? Did you do any purification? Did you enrich for anything (e.g. a special PTM)? Do you want to identify endogenously digested peptides? How were the proteins/peptides extracted and in what form are they delivered (protein or already digested). In which buffer do you submit your samples (Buffer ID from Section 1.5.)? Indicate the volume of the respective sample in the Vol. column and write down the protein or peptide concentration in the “Conc” column.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **ACE ID** | **Your ID** | **Organism** | **Organ/ cell line** | **Treatment/ experimental setup** | **Buffer ID** | **Vol. [µL]** | **Conc. [µg/µL]** |
| FK01 | FAK\_0704\_A1 | mouse | lung | Thorax-irradiated (X‑Ray) 10 Gy, proteins were ex­tracted in B#\_01. Concentration was determined by Bradford assay. Proteins are not digested. | B#\_01 | 500  | 2.11 |
| FK02 | FAK\_0704\_B1 | ARA | leaf | Wildtype *A. thaliana* (ARA), not treated. | B#\_03 | 10 | 1 |
| FK03 | FAK\_0666\_A1 | human | Recomb. protein | 1 µg purified DegS protease (overexpressed in *E. coli*) was incubated with its substrate XYZ in B#\_01 and incubated for 2h.  | B#\_03 | 10 | 1 |
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Add rows if necessary

## 1.7. Sample preparation for MS

We assume that after you individually treated your samples (see Section 1.6.) you performed all subsequent steps identically (sample preparation for MS). If this is not the case you have to submit separate forms!

### How did you isolate proteins after the treatment described in Section 1.6.?

Write how you prepared the protein extracts. Use Buffer ID’s from Section 1.4.

 Your text

### How did you determine the protein and peptide concentration?

For proteins e.g. Bradford assay, for peptides “NanoDrop at 280 nm” or “estimate based on protein concentration”.

 Your text

### Do you deliver peptide or protein?

 Your text

### Which strategy did you use to generate your peptides?

In-gel digestion (IGD); in-solution digestion (ISD); on-bead digestion (OBD).

 Your text

### How did you denature your proteins before reduction and alkylation? Did you reduce and alkylate your peptide samples and how was this done?

Write down how you denatured your proteins … did you use Urea, SDS or something else? Elevated temperatures? Indicate the alkylating agent (e.g. IAM) and if it is an obscure modifying agent the mass it introduces.

 Your text

### Did you perform a StageTip purification of your peptide samples?

Please note: StageTip purification is obligatory for all MS samples. If you indicate “no” here we will do the Stage Tip purification by default.

 Your text

### Did you enrich for a special PTM (e.g. phosphorylated peptides; indicate which samples)?

 Your text

## 1.8. Gel images or other images/schemes

Please provide any schemes or gel images that may help to understand the experiment you did. Just cut and paste the image below. If you deliver gel pieces or samples derived from IGD you **must** deliver the gel image before you cut out the gel slices. Please indicate which region corresponds to which sample (ACE ID) and indicate which stain or detection procedure you used (e.g. Coomassie or fluorescence detection, chemiluminescence).

Your image/images

## 1.9. Protein Sequences

Please deliver below the exact protein sequences of any recombinant proteins used in your experiment. Use the FASTA format and indicate tags (HIS, FLAG, any additional sequence) and mention deletions or any other deviation from wild type.

>WT\_HTRA1| C-term HIS tag

MGQEDPNSLRHKYNFIADVVEKIAPAVVHIELFRKLPFSKREVPVASGSGFIVSEDGLIVTNAHVVTNKHRVKVELKNGATYEAKIKDVDEKADIALIKIDHQGKLPVLLLGRSSELRPGEFVVAIGSPFSLQNTVTTGIVSTTQRGGKELGLRNSDMDYIQTDAIINYGNSGGPLVNLDGEVIGINTLKVTAGISFAIPSDKIKKFLTESHDRQAKGKAITKKKYIGIRMMSLTSSKAKELKDRHRDFPDVISGAYIIEVIPDTPAEAGGLKENDVIISINGQSVVSANDVSDVIKRESTLNMVVRRGNEDIMITVIPEEIDPRSLEHHHHHH

Your sequences

## 1.10. Protein Databases

If you do not specify a database we will use a standard Uniprot database installed on our server. If you are working with an obscure non sequenced organism you must indicate which database fits your organism best.

Your Text

## 1.11. What can we do for you?

If you have a clear idea what you want us to do for you, please feel free to write it down. I will check what you want us to do. If I have questions I will contact you. You may also contact me either by mail or by phone: +49 201 183 4294 to discuss your project and possible approach routes to your goal. Please check also the ACE website. You will find a description of possible analysis types established at the ACE (coming soon).

Your text

# 2. ACE contributors

* My text

# 3. Sample preparation

This section contains information about the sample preparation. All steps undertaken by us to generate MS samples are documented here.

My text

# 4. LC settings

In this sub-section you will find all the details about the LC system (column length, particle diameter, pore size, column type, inner diameter, gradient, solvents used, retention time reproducibility and general performance of the system). Since these values only change every 2 month or so they are already pre-inserted. The only thing that changes is the gradient.

|  |  |
| --- | --- |
| MS device | Thermo Orbitrap Fusin Lumos / Elite |
| LC device | Thermo Easy-nLC 1000 |
| ion source | Thermo Nanospray Flex  |
| **Analytical column** | Self-packed fused silica capillary with integrated pico frit emitter; New Objectives PF360-75-15-N-5 |
| column diameter | Length (LC) = xx cm; ID = 75µm; OD = 360 µm; emitter 15 µm |
| stationary phase | Reprosil-Pur 120 C18-AQ, Dr. Maisch GmbH  |
| particle diameter (dp) | x µm |
| Pore size | 120 Å |
| Column ID | ACxx |
| Column oven | Sonation column oven PRSO-V1 |
| Column oven temp. | 45°C |
| **Pre column** | - |
| column dimensions | - |
| stationary phase | - |
| particle diameter (dp) | - |
| pore size  | - |
| **solvents** | A: 0.1% FA in UPLC waterB: 0.1% FA in UPLC ACN |
| gradient | These parameters are experiment dependent and will be inserted once the experiment is done |
| retention time re­producibility | Between ±0.08 and 0.15 min, based on average retention time shift of 4 monitored BSA peaks. To put this into perspective: LFQ calculation in MaxQuant requires rt shift <0.7 min. |
| System performance | Overall good |

# 5. MS sequence and settings

## 5.1. Sequence

* END

## 5.2. Settings

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Project** | **MS** | **general** | **MS1** | **MS2** | **MS2** | **MS3** | **Comments; special settings** |
| ACE\_0608 | Lumos | Tune v3.3.2782.28Gradient: 200 min | Analyzer: FTRes.: 120000 SR: 375 - 1500AGC: StandardAcT: AutoRF: 30SF: --DDM: CT/2sec | Analyzer: FTRes./ScR: 15000/-SR: AutoAGC: StandardAcT: AutoCS: +2 to +7IsM: QIsW: 1.6 Frag.: HCDNCE: 30 |  |  | classic orbitrap experiment: MS1 in Orbitrap at high resolution and data dependent MS2 in Iontrap at turbo scan rate. Dynamic exclusion enabled (exclude after n times=1; Exclusion duration (s)= 60; mass tolerance= ± 10ppm) |

Note: **FT**= Fourier Transform (Orbitrap); **IT**= Iontrap; **Q**= Quadrupol; **Res.**= max. Resolution at 200 m/z (Lumos) or 400 m/z (Elite) [FWHM (full width at half maximum)]; **ScR**= scan rate for measurements in the IT; **SR**= scan range [m/z]; **AGC**= automatic gain control, max number of acquired ions per measurement; **AcT**= max. Ion acquisition time [ms]; **CS**= charge states used for fragmentation; **IsM**= Isolation mode (Q or IT), MS2 isolation and further is only done in IT; **IsW**= Isolation window [m/z], value followed by scan mode the isolation is based on (MS1, MS2 …) **Frag.**= Fragmentation method; **HCD**= Higher-energy collisional dissociation; **CID**= Collision-induced dissociation; **ETD**= Electron-transfer dissociation**; EThcD=** Electron-Transfer/Higher-Energy Collision Dissociation; **sHCD**= stepped HCD**; NCE**= normalized collision energy; **cycles**: number of MSn recorded or max cycle time; RF= RF Lens [%]; **SF**= Source Fragmentation [V]; **DDM**: Data dependent Mode (cycle time in seconds, CT/[s] or number of scans, NS); **NS**= Number of data dependent scans

# 6. Data Quality Assessment

This section contains information about quality of the MS runs and the RAW files. We currently use RawMeat, Xcalibur and PREVIEW for quick quality assessment.

* My text

# 7. Database Search & Data Analysis

Here we describe how we processes the MS-RAW files, which programs and databases are used. In certain cases we also do statistical evaluation. This Section will often contain subsections. Each subsection represents a different database search & data analysis approach which delivers individual result files. Occasionally subsections will also contain correspondence with collaborators (e.g. request for different search …).

## 7.1. Data Analysis 1

|  |  |
| --- | --- |
| Program & version | MaxQuant v1.5.5.30. |
| Search engine | Andromeda  |
| settings | Basically default; LFQ and MBR were turned on |
| Static modification | Carbamidomethyl (C) |
| Digestion mode | Trypsin/P (specific), 2 missed cleavages |
|  |  |
| Dynamic modification | Acetyl (N-term); Oxidation (M) |
| Modification included in quantification | Oxidation (M) |
| Databases | 1. Contaminants
2. ACE\_0056\_rep\_prot\_file\_v01.fasta
 |
| Annotation | ACE\_0056\_annot.Nibenonly\_new.txt |

* My text

## 7.2. Data Analysis 2

|  |  |
| --- | --- |
| Program & version | MaxQuant v1.5.5.30. |
| Search engine | Andromeda  |
| settings | Basically default; LFQ and MBR were turned on |
| Static modification | Carbamidomethyl (C) |
| Digestion mode | Trypsin/P (specific), 2 missed cleavages |
|  |  |
| Dynamic modification | Acetyl (N-term); Oxidation (M) |
| Modification included in quantification | Oxidation (M) |
| Databases | 1. Contaminants
2. ACE\_0056\_rep\_prot\_file\_v01.fasta
 |
| Annotation | ACE\_0056\_annot.Nibenonly\_new.txt |

* My text

# 8. Conclusion

# 9. Appendix

This part of the report contains the LC and MS settings for the different approaches.

## 9.1. RawMeat

Below you will find several screenshots from the program RawMeat (http://vast-scientific.software.informer.com/). We use this program to evaluate several critical parameters of MS runs. If nothing is written under the images all seems to be OK. If there are abnormalities or deviations from the standard there will be a comment.

### 9.1.1. General Data

### 9.1.2. Scans

### 9.1.3. Chromatogram

### 9.1.4. Charges

### 9.1.5. TopN-Spacing

### 9.1.6. Time Effects

## 9.2. RawBeans

## 9.3. Xcalibur Chromatograms

### 9.3.0. PSM’s and sequence coverage BSA Samples

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Score | coverage [%] | unique pep | #PSM | r.t. peak 1 | r.t. peak 2 | r.t. peak 3 | r.t. peak 4 |
| BSA before  |  |  |  |  |  |  |  |  |
| BSA in between  |  |  |  |  |  |  |  |  |
| BSA in between |  |  |  |  |  |  |  |  |
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| BSA in between |  |  |  |  |  |  |  |  |
| BSA in between |  |  |  |  |  |  |  |  |
| BSA after  |  |  |  |  |  |  |  |  |

Note:

r.t. = retention time

### 9.3.1. BSA before samples

### 9.3.2. BSA in between

### 9.3.3. BSA in between

### 9.3.4. BSA in between

### 9.3.5. BSA after

## 9.4. Skyline

## 9.5. Preview

## 9.6. Miscellaneous